ock–initial segment spike had increased from 0.56 msec to 0.84 msec (Fig. 2, B2). This result might be explained if, due to preferential rostral migration of the drugs, they did not reach active zones of the axon. This possibility is unlikely since the axon narrows as it approaches the soma, and, thus, rostral diffusion would be expected to be restricted. In order to be certain that the injected drug had access to active zones of the axon, we penetrated M axons slightly distal to the first active zone (Fig. 2, C and D). After injection of both TEA and 4AP (450 nA/min) into the axon, the duration of the isolated axon spike remained unchanged (Fig. 2, C1 versus C2). The duration of the axon hillock–initial segment spike had, however, increased from 0.54 msec to 0.78 msec (Fig. 2, D1 versus D2). As the location of the first axonal active zone was probably between the injection site and the axon hillock–initial segment, it is very unlikely that the lack of effect of TEA and 4AP on the isolated axon spike resulted from failure of the drugs to reach that first axonal active site.

The distribution along the Mauthner axon of voltage-sensitive channels blocked by TEA and 4AP differs from what would be expected on the basis of most models of axonal action potential electrogenesis, which generally include voltage-sensitive potassium channels at each active site. Our data indicate that, in the axon, potassium channels sensitive to TEA and 4AP are restricted to the axon hillock–initial segment region and do not occur at active zones along the myelinated axon. It remains possible that voltage-sensitive channels, resistant to blockade by both TEA and 4AP, occur along the M axon. This possibility, if demonstrated experimentally, would be the first case of a cell's having two classes of voltage-sensitive potassium channels, with one being completely insensitive to both TEA and 4AP. Alternatively, the kinetics of voltage-sensitive potassium channels in the axon might be too slow to contribute to impulse repolarization. It seems much more likely, however, that active zones along the myelinated M axon generate action potentials much as nodes of Ranvier along rat and rabbit sciatic nerve fibers and rat dorsal column axons do (*4, 5*)—that is, without a conductance increase to any specific ion playing a role in repolarization (*12*). This interpretation of these data suggests that two mechanisms of action potential electrogenesis, having one or two active membrane conductance changes, arose early in the evolutionary history of myelinated axons; the interpretation also raises questions about the distribution of these two physiological types in vertebrate nervous systems.

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9. Electrodes contained the following solutions: 1*M* KCl and 2*M* TEA; 2.7*M* KCl and 0.1*M* 4AP; or 1*M* KCl, 2*M* TEA, and 0.1*M* 4AP. Electrodes with both drugs were used since some voltage-sensitive potassium channels in *Aplysia* are blocked only by 4AP and TEA together [M. Klee, *Brain Res. Bull.* **4**, 162 (1979)].
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11. Width at half-amplitude was the measure of duration of somatically recorded action potentials, and the time between positive and negative peaks of the differentiated record was measured as the duration of axonally recorded action potentials.
12. Two other possible indicators of voltage-sensitive potassium conductances would be a rectification in the axonal current-voltage relationship and an undershoot after the impulse. Rectification did not occur, but we have occasionally observed an undershoot associated with a conductance increase, the mechanism of which remains to be elucidated.
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Chemical Modification of Carotid Body Chemoreception by Sulfhydryls

Abstract. *Sulfhydryl reagents cause striking augmentation of the chemoreceptor responses of the carotid body to hypoxia. This indicates that a cellular plasma membrane protein with a reactive sulfhydryl group is a constituent part of the chemoreceptor architecture and provides a means of identification, localization, and isolation of the protein.*

Chemoreception of O_2 in the carotid body and generation of action potentials in the chemoreceptor afferents presumably require several steps, including detection, feedback regulation, and energy transduction. Even though our understanding of the nature of O_2 chemoreception is incomplete, it seems clear that specific components, not shared generally by all cells, are common to cellular processes that participate in sensory mechanisms. Since cell membrane–bound systems with active sulfhydryl groups are part of the receptor architecture in several sensory systems (*1–5*), similar systems may operate in the chemoreception processes of the carotid body. Evidence for the participation of a membrane-bound system in the expression of chemoreception is indicated by the interaction of O_2 with dopamine (*6*) and isoproterenol (*7*), because the receptors for these catecholamines are localized on the cell membranes (*2, 3*).

If a membrane protein whose active site contains sulfhydryl groups participates in the expression of chemoreception, chemical modification of the plasma membrane protein might cause specific alterations in chemoreception. This hypothesis was tested by treating the carotid body with *p*-chloromercuriphenylsulfonic acid (PCMBS), which does not penetrate the plasma membrane, and with the alkylating agent, *N*-ethylmaleimide (NEM), which does penetrate the plasma membrane. The hypoxic response was selectively augmented by PCMBS and this effect of PCMBS was saturable, suggesting that some component of the receptor architecture is a protein with active sulfhydryl sites.

The discharge rates of the carotid body chemoreceptor afferents were studied in six cats that were anesthetized with Nembutal (30 mg/kg), paralyzed with gallamine (3 mg/kg-hour), and artificially ventilated at a maintained rectal temperature of 38°C (*8*). The external and lingual arteries were ligated and a fine arterial cannula was inserted just upstream from the carotid body for the administration of PCMBS and NEM. Chemoreceptor afferents were isolated and identified from the peripheral end of a cut sinus nerve (*8*). Airway PO_2 and PCO_2, arterial blood pressure, and carotid chemoreceptor activity were recorded continuously. The responses of a single or a few carotid body chemoreceptors to steady-state levels of P_aO_2 (30 to 500 torr) at a constant P_aCO_2 were investigated before and after several doses of PCMBS. While the cat was ventilated

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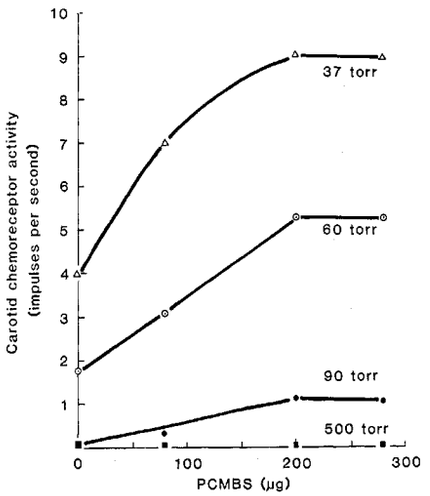

Fig. 1. Carotid chemoreceptor activity in one cat at four levels of P_aO_2, and constant P_aCO_2 (22 to 23 torr) and pH (7.470 to 7.480), before and after three successive intra-arterial injections of PCMBS. The PCMBS augmented the response, which was saturated at a dose of about 200 μg.

with room air, PCMBS (500 μg per milliliter of saline) was administered by close intra-arterial injections (40 to 200 μg) to the carotid body. The injections of PCMBS were followed by an increased chemoreceptor discharge rate, which became stable in a few minutes and persisted for several hours. Thus the PCMBS doses were cumulative. After each dose, the responses of the carotid body chemoreceptors to hypoxia were measured. The sequence of measurements was from normoxia to successive levels of hypoxia and then to hyperoxia. Each P_aO_2 was maintained for about 3 minutes in order to achieve a steady state. In this manner, all O_2 tests were completed in 15 minutes. After the PCMBS tests were completed, NEM administered intra-arterially in two or three small doses (4 to 16 μg) caused a further persistent stimulation of the chemoreceptors. The NEM effects were also cumulative. After each dose the responses to hypoxia were recorded as before. Arterial blood samples (about 1 ml) were taken anaerobically during each steady state, and PO_2, PCO_2, and pH were measured at 38°C. The chemoreceptor activity was plotted against P_aO_2 to assess the effects of PCMBS and NEM. Similar results were obtained in all of the experiments.

Three striking features of the PCMBS effect on the carotid chemoreceptor are (i) an augmented response to hypoxemia, (ii) saturation of the augmented response, and (iii) a virtual lack of stimulation of the chemoreceptors during hyperoxia (P_aO_2 ~ 500 torr) (Fig. 1). The control response to successive levels of hypoxia was characteristically hyperbolic (Fig. 2). After 200 μg of PCMBS, the response to successive levels of low P_aO_2 was augmented. A second dose of 200 μg of PCMBS caused no further stimulation. After this saturation dose of PCMBS, NEM caused further stimulation of chemoreceptor activity. The stimulatory effects appeared to be similar in magnitude at each level of P_aO_2 without hypoxic augmentation.

The chemoreceptor afferents were also stimulated when NEM was administered before PCMBS, and the effect was augmented by hypoxia. The augmentation resembled that caused by PCMBS. When administered after PCMBS, NEM showed an additive effect only. Thus the NEM effect included that caused by PCMBS. This effect seems to be a selective modification of chemoreceptor activity, being saturable at any P_aO_2 and augmented by hypoxia. Since PCMBS is ionized at the normal plasma pH, it does not readily permeate the cell membrane (*4*). Since the membrane is the first cellular element to encounter the agent, ligand binding occurs at the cell surface first. The saturation effect indicates that all the accessible ligand-sensitive sites were bound and that the PCMBS reaction was selective.

The stimulating effect of NEM, which easily penetrates cell membranes (*4*), could have been due to binding with membrane proteins that are not accessible to PCMBS, as well as to the reactions with the intracellular metabolic enzymes that are associated with the excitation process (*9–12*). Additional amounts of NEM (16 μg) caused a burst of chemoreceptor activity followed by complete cessation of activity, which could be due to a conduction block similar to that of the squid axon response (*13*).

The interaction of these sulfhydryl agents in low concentrations with O_2 chemoreception indicates that sulfhydryls are a component of the chemoreceptor architecture. The sulfhydryl ligand appears to be a critical part of the transduction protein, and it is perhaps this protein which ultimately gives rise to the stimulus interaction of O_2 and CO_2 (*8*).

The effect of PCMBS on O_2 chemoreception in the carotid body resembles that of haloperidol (*6*), which binds to dopamine receptors (*2*). Accordingly, one plausible interpretation is that PCMBS and haloperidol bind to protein receptors to induce a similar effect. The information that a membrane protein binds sulfhydryls and participates in the expression of O_2 chemoreception might be useful in determining the precise identification of the membrane protein and its localization on the structural components of the carotid body.

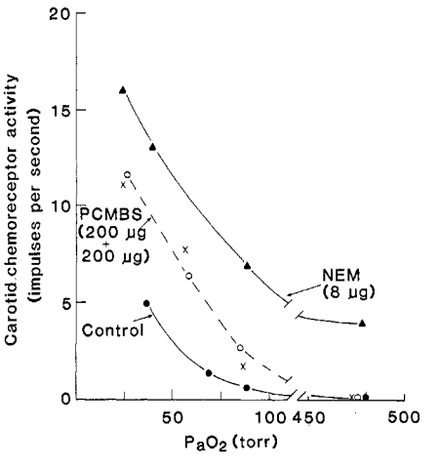

Fig. 2. Carotid chemoreceptor responses to hypoxia at constant P_aCO_2 before and after successive intra-arterial administration of PCMBS and NEM. After the saturation dose of PCMBS (200 μg + 200 μg), NEM caused an additional stimulation of the chemoreceptors. The magnitude of the latter effect was similar at all levels of P_aO_2.

Since mercurials are used as therapeutic agents (*14*), the mercurials that augment the chemoreceptor response to hypoxia might be useful for therapeutic application in cases where the chemoreflex response to hypoxia is blunted (*15*).

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